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(54) Title: POLYENE MACROLIDE PRE-LIPOSOMAL POWDERS

(57) Abstract

The present invention involves a process for producing fine powder suitable for the preparation of antifungal polyene macrolide-containing liposomes upon suspension in an aqueous solution. This process comprises the following steps. Quantities of polyene macrolide and phospholipids are dissolved respectively in a first solvent and a second solvent to form a first solution and a second solution. The first solution and the second solution are mixed in a desired ratio to form a mixture. The first solvent and the second solvent are then removed from the mixture, for example by evaporation, to form a residue. The residue is then dissolved in a third solvent comprising tertiary butanol and methylene chloride to form a third solution. The third solvent is then removed from the third solution to form a remnant. The remnant is then dissolved in a solvent consisting essentially of tertiary butanol to form a fourth solution. The fourth solution is then filtered through a filter having orifices of between about 0.05 and 0.5 micrometers in diameter to produce a filtrate. The filtrate is lypohilized to remove the tertiary butanol and a fine powder remains. This fine powder may be used to form polyene macrolide-containing liposomes by simple incubation or suspension in an aqueous solution.

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Polyene macrolide pre-liposomal powders.

BACKGROUND OF THE INVENTION

The present invention relates to a composition of matter usable to form liposomes comprising antifungal polyene macrolides and the production thereof.

Clinical observations and animal experimental studies have added to the understanding of host-fungal interactions. It is becoming recognized that host defense against fungal disease is multifactorial and may vary, depending on the etiologic agent. The mechanisms of resistance are not well defined in most instances, but various innate barriers and cell-mediated immune responses seem to be of primary importance. Clearly, debilitation of innate defenses and of cell-mediated immune responses can increase an individual's susceptibility to severe fungal disease from opportunistic agents such as Cryptococcus neoformans and species of Candida and Aspergillus, as well as from fungal pathogens such as Histoplasma capsulatum and Coccidioides immitis. The difficulty in gaining a complete understanding of the critical host defenses has been further complicated by many studies that show fungi may affect various host immune functions adversely. Although it is too early to evaluate the clinical importance of many of these experimental findings, investigators have demonstrated that fungi impair neutrophil function, induce IgE responses, and cause suppression of cell-mediated immune responses.

Host changes likely to be associated with increased susceptibility may be accidentally induced, as in traumatic injuri s (such as burns or puncture wounds); self-induced, as in chronic alcoholism; naturally occurring, as in diabetes mellitus, various congenital immune deficien-

cies, collagen diseases, lymphoreticular neoplastic disease, and other types of tumors; or iatrogenically induced by instrumentation (such as catheterization), surgical procedures (such as open heart surgery), or by use of cytotoxic drugs (as in an attempt to prevent graft rejection and to treat neoplastic disease), corticosteroid therapy, and long-term use of broad-spectrum antibiotics.

Chemical factors that aid resistance to fungal diseases are poorly defined. Knowledge of these substances is based primarily on circumstantial evidence at the clinical level and in vitro observations at the experimental level. Hormonally associated increases in lipid and fatty acid content on the skin occurring at puberty have been correlated with increased resistance to tinea capitis caused by the dermatophyte Microsporum audouinii, although pubescent changes are not the sole factors in resistance. Substances in serum, cerebrospinal fluid, and saliva may limit growth of Cryptococcus neoformans, and basic peptides in body fluids have been shown to inhibit Candida albicans.

Results of clinical and experimental studies indicate that C. albicans, C. neoformans, Aspergillus fumigatus, and C. immitis activate the alternative pathway of the complement cascade. Because of the polysaccharide nature of fungal cell walls, it is expected that all medically important fungi activate complement. Such activation may be important in defense against some mycoses; a positive correlation has been demonstrated between animals deficient in late-acting complement components (C3-C9) and increased susceptibility to fungi such as C. neoformans and C. albicans. Assuming that phagocytic cells are important in resistance to fungi, complement activation

may play a role by provoking an acute inflammatory response on generation of complement fragments C3a and C5a, and by coating the fungal elements with opsonic fragments C3b and C3d for ingestion by phagocytic cells.

The systemic mycoses of humans and other animals are caused by some fungi that are pathogenic and cause disease in the healthy host, and by other fungi (opportunistic pathogens) that are usually innocuous but cause disease in patients whose immune defenses are impaired. Some of these fungi may be saprophytes in nature (soil, bird droppings), whereas others are a part of the normal human flora (commensals). In no case are humans the solitary or necessary host.

An example of a soil saprophyte is Histoplasma capsulatum, which commonly causes infection in endemic areas; 80%-90% of adults react positively to histoplasmin in delayed cutaneous hypersensitivity tests. An example of an opportunistic pathogen is Candida albicans, normally present in the oral cavity, gastrointestinal tract, and probably the skin. In the patient with acute leukemia, however, C. albicans is commonly present in blood, causing a fulminant, usually fatal, septicemia. Other opportunistic infections are seen in patients with diabetic acidosis (mucormycosis) and Hodgkin's disease (for example, cryptococcosis and histoplasmosis). The pathogenesis of these mechanisms is obscure, but cell-mediated immunity seems to be essential for a good prognosis.

Neither active vaccines nor passive immune serum immunization has been sufficiently successful to result in commercially available preparations.

Treatment of active disease may be symptomatic (for example, pain relief), sometimes surgical (resection of irremedially damaged tissue and correction of hydrocephalus), and, most successfully, chemotherapeutic (Table 1). Among the chemotherapeutic agents commonly used are hydroxystilbamidine isethionate, amphotericin B, 5-fluorocytosine (Flucytosine), miconazole, and ketoconazole. Response to these drugs varies according to the fungus, type of disease, and course of illness. For example, response is good in most <u>B. dermatitidis</u> infections, but is poor in most diseases caused by <u>A. fumigatus</u>. Response is better for skin lesions caused by <u>B. dermatitidis</u> than for meningitis due to <u>C. immitis</u>; response is better in chronic cryptococcosis than in fulminant candidiasis. Table 1 shows a listing of some systemic mycoses and generally accepted chemotherapeutic agents.

TABLE 1

CHEMOTHERAPEUTIC AGENTS FOR SYSTEMIC MYCOSES

Disease	First Choice	Second Choice
Aspergillosis	Amphotericin B	Ketoconazole
Blastomycosis	Amphotericin B	Hydroxystilbamidine isethionate
Candidiasis	Amphotericin B	Flucytosine or ketoconazole
Coccidioidomycosis	Amphotericin B	Ketoconazol
Cryptococcosis	Amphotericin B Flucytosine	Either drug alone*
Histoplasmosis	Amphotericin B	Ketoconazole*
Mucormycosis	Amphotericin B	Miconazole*
Paracoccidioidomycosis	Amphotericin B	Sulfonamides, Ketoconazole*

*Depending on minimal inhibitory concentration necessary for the fungus.

Infection is the cause of death in 51% of patients with lymphoma and 75% of patients with leukemia. Although bacteria are the causative organisms of many such infections, fungi account for 13% of the fatal infections in patients with lymphoma and for more than 20% of patients with leukemia. The fungus Candida albicans causes more than 80% of these infections, and Aspergillus spp. is also a frequent cause of such infections. In addition, fungal infection is a major cause of morbidity and mortality in patients with congenital and acquired deficiencies of the immune system. Much concerted effort has been expended in search of agents useful in treating fungal infections f humans. As a result, many compounds have been isolated and shown to have antifungal activity, but problems associated with solubility, stability, absorption, and toxicity have limited the therapeutic value of most of them in human infections. The most useful antifungal antibiotics fall into one of two categories: those that affect fungal cell membranes and those that are taken up by the cell and interrupt vital cellular processes such as RNA, DNA, or protein synthesis. Table 2 lists some useful antifungal agents and their mechanisms of action.

TABLE 2

SOME USEFUL ANTIFUNGAL AGENTS, THEIR CHEMICAL CLASSIFICATION, AND THEIR MECHANISMS OF ACTION

Class	Compounds	Mechanism
Polyene	Amphotericin B Nystatin	Interacts with sterols (ergosterol) in fungal cell membrane, render ing cells selectively permeable to the outfl of vital constituents, e.g. potassium
Imidazole	Miconazole Clotrimazole Ketoconazole	Inhibits demethylation of lanosterol thus preventing formation c ergosterol, a vital component of fungal ce membrane; also has a direct cidal effect on fungal cells
Pyrimidine	5-Fluorocytosine	Is taken up and deaminate by susceptible cell tc form 5-fluorouracil, which in turn inhibits RNA synthesis; also thought to inhibit thymidylate synthetase and DNA synthesis
Grisan	Griseofulvin	Binds to tubulin and inhibits microtubule assembly
3-Arylpyrrole	Pyrrolnitrin	Appears to inhibit termin electron transport between succinate or NADH and coenzyme Q
Glutaramide	Cycloheximide	Inhibits protein synthesi at 80S ribosomal level preventing transfer of aminoacyl tRNA to the ribosome

The polyene macrolide antibiotics are secondary metabolites produced by various species of Streptomyces. Several common features of these compounds are useful in classifying the more than 80 different polyenes that have been isolated. All are characterized by a macrolide ring, composed of 26-38 carbon atoms and containing a series of unsaturated carbon atoms and hydroxyl groups. These features of the molecule contribute to the polyenes' amphipathic properties (those relating to molecules containing groups with different properties, for example, hydrophilic and hydrophobic). The ring structure is closed by the formation of an internal ester or lactone bond (Figure 1). The number of conjugated double bonds vary with each polyene, and the compounds are generally classified according to the degree of unsaturation.

Toxic effects of polyene macrolides appear to be dependent on binding to cell membrane sterols. Thus, they bind to membranes of fungus cells as well as to those of other eukaryotic cells (human, plant, and protozoa), but not to bacterial cell membranes, which do not contain membrane sterols. The interaction of polyene macrolides with mammalian and fungal membrane sterols results in transmembrane channels that allow the leakage of intra-cellular components leading to cell deaths.

The usefulness of an antibiotic is usually measured by the differential sensitivity of the pathogen and host. Two polyene macrolides agents, nystatin and amphotericin B, are relatively specific for fungi and have thusfar proven to have therapeutic usefulness in humans. The relative specificity of these two polyene macrolides may be based on their greater avidity for ergosterol, the

principal sterol of fungal membranes, compared to cholesterol, the principal sterol of human cell membranes.

Amphotericin B is a heptaene macrolid with seven resonating carbon bonds. The compound was first isolated from broth filtrates of S. nodosum in 1956. Like other polyene macrolide antibiotics, amphotericin B is insoluble in water. The problem of its solubility has been circumvented by combining the antibiotic with sodium deoxycholate and sodium phosphate and hydrating the mixture with sterile water or saline. Amphotericin B is the polyene antibiotic thusfar most sufficiently nontoxic to humans that it has been used parenterally at effective doses against various fungi.

Nystatin, first isolated from S. noursei, is structurally related to amphotericin B, but is not classified as a heptaene because the conjugated portion of the ring is interrupted and thus forms a tetraene and a diene. Tolerated well both orally and topically, the drug is not available for intravenous use because of its presumed high toxicity and aqueous insolubility. Nystatin is available as oral tablets (500,000 units) or as an ointment for topical use (100,000 units/g). It is used in the management of cutaneous and mucocutaneous candidiasis.

It has recently been shown that the encapsulation of certain drugs in liposomes before administration to the patient can markedly alter the pharmacokinetics, tissue distribution, metabolism and therapeutic efficacy of these compounds. Liposomes may be defined as lipid vesicles which are formed spontaneously on addition of an aqueous solution to a dry lipid film. Further, the distribution and pharmacokinetics of these drugs can be modified by

altering the lipid composition, size, charge and membrane fluidity of the liposome in which they are encapsulated.

Recently, liposomes hav been used as carriers of amphotericin B for treatment of murine leishmaniasis (New, R.R.C., et al., "Antileishmanial Activity of Amphotericin and Other Antifungal Agents Entrapped in Liposomes." J. Antimicrob. Chemother., Vol. 8 (1981), pp. 371-381), histoplasmosis (Taylor, R.L., et al., "Amphotericin B in Liposomes: A Novel Therapy for histoplasmosis." Am. Rev. Respir. Dis., Vol. 125 (1982), pp. 610-611), cryptococosis (Graybill, J.R., et al., "Treatment of Murine Cryptococosis with Liposome-Associated Amphotericin B." J. Infect. Dis., Vol. 145 (1982), pp. 748-752). and candidiasis (Tremblay, C., et al., "Comparative Efficacy of Amphotericin B (AMB) and Liposomal AMB (lip-AMB) in Systemic Candidiasis in Mice." Abstr. 1983 ICAAC, No. 755 (1983), p. 222). Liposome-encapsulated Amphotericin B has also been used for treatment of coccidioidomycosis in the Japanese macaque (Graybill, J.R., et al., "Treatment of Coccidioidomydosis (cocci) in Primates Using Liposome Associated Amphotericin B (Lipo-AMB)." Abstr. 1982 ICCAC, No. 492 (1982), p. 152).

The treatment of fungal infections remains a major problem in spite of the availability of effective antifungal drugs such as the polyenes. Most of the available polyene antibiotics have toxic side effects that limit their clinical application. Nystatin, a tetraene-diene polyene macrolide antibiotic, has high hydrophobicity, which has precluded its effective systemic administration. It has been used as suspensions prepared in various ways and administered to the patients orally. However, thes studies have generally failed to document a beneficial

effect of nystatin administration against systemic fungal infections.

Th present inventors have recently demonstrated that liposome-encapsulated amphotericin B may be used to tr at experimental murine candidiasis (Lopez-Berestein et al., J. Infect. Dis., Vol. 150, pp 278-283 (1984) and in the treatment of fungal infections in patients with leukemia and lymphoma (Lopez-Berestein et al., J. Infect. Dis., Vol. 151, pp 704-71- (1985).

SUMMARY OF THE INVENTION

The present invention involves a process for producing fine powder suitable for the preparation of antifungal polyene microlide-containing liposomes upon suspension in an aqueous solution. This process comprises the following steps. Quantities of polyene macrolide and phospholipids are dissolved respectively in a first solvent and a second solvent to form a first solution and a second solution. The first solution and the second solution are mixed in a desired ratio to form a mixture. The first solvent and the second solvent are then removed from the mixture, for example by evaporation, to form a residue. The residue is then dissolved in a third solvent comprising tertiary butanol and methylene chloride to form a third solution. The third solvent is then extracted by evaporation from the third solution to form a remnant. The remnant is then dissolved in a solvent consisting essentially of tertiary butanol to form a fourth solution. The fourth solution is then filtered through a filter having orifices of between about 0.05 and 0.5 micrometers in diameter to produce a filtrate. The filtrate is

lyophilized to remove the tertiary butanol and a fine powder remains. This fine powder may be used to form polyene macrolide-containing liposomes by simple incubation or suspension in an aqu ous solution.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

A stable powder suitable for the direct preparation of liposome-incorporated antifungal polyene macrolides may be made by a process of the present invention. While the conditions described herein are specifically applicable to nystatin and amphotericin B, other polyene macrolide antifungals may be likewise used, but with minor modifications of procedure apparent to those skilled in the art upon a minimal amount of experimentation.

The process for pre-liposomal polyene macrolide powder formation of the present invention involves dissolution of an antifungal polyene macrolide such as nystatin or amphotericin B in a first organic solvent such as methanol to form a first solution. Phospholipids are dissolved in a second organic solvent such as, for example, chloroform, to form a second solution. The first solution and the second solution are mixed to form a first mixture having a ratio of antifungal polyene macrolide to phospholipid between about 1:5 and about 1:50, preferably of about 1:10. The organic solvents are removed from the mixture, for example, by solvent evaporation under reduced pressure and at a temperature between about 35°C and about 45°C, until a residue such as a dry film is formed. The residue is then dissolved in a quantity of a third organic solvent such as a mixture of tertiary butanol and methylene chloride in a ratio between about 2:1 (preferred for

nystatin) and about 1:40 (preferred for amphotericin B) and the solvent evaporated to leav a r mnant. The remnant is dissolved in a solvent consisting essentially of tertiary butanol to form a fourth solution which is warmed, if necessary for clarification, and passed through a filter having orifices of between about 0.05 and 0.5 micrometers (um) in diameter. If warming is desired to clarify the fourth solution, particularly with amphoterecin B, the warming is preferably to a temperature between about 50°C and about 70°C. The filtrate is subjected to freezing, for example, with dry ice-acetone. The frozen material is then lyophilized until essentially all solvent is removed. After lyophilization, a fine pre-liposomal polyene macrolide powder is produced. This powder is readily and stably stored under commonly available dry and cool storage conditions.

The above-described pre-liposomal polyene macrolide powder may be easily used to reconstitute a liposome suspension according to the following general procedure. The powder is added to an aqueous solution such as pyrogen-free saline, and allowed to incubate at 25°C to 45°C for 1-10 minutes for a liposome suspension to form. Polyene macrolide content may be measured by dissolution of the liposomes in methanol and monitoring of optical density at a wavelength characteristic for polyene macrolide absorption.

Representative, suitable phospholipids in the present invention are phosphatidylcholine, both naturally occurring and synthetically prepared, phosphatidic acid, phosphatidylserine, phosphatidylethanolamine, sphingolipids, phosphatidyglycerol, spingomyelin, cardiolipin, glycolipids, gangliosides, cerebrosides and the like used

either singularly or intermixed such as in soybean phospholipids.

More particularly useful phospholipids include egg phosphatidylcholine, dilaurylphosphatidylcholine, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, 1-myristoyl-2-palmitoylphosphatidylcholine , 1-palmitoyl-2-myristoyl phosphatidylcholine, 1-stearoyl-2-palmitoyl phosphatidylcholine, dioleoylphosphatidylcholine, dilauryloylphosphatidylglycerol, dimyristoylphosphatidylglycerol, dipalmitoylphosphatidylglycerol, distearoylphosphatidylglycerol, dioleoylphosphatidylglycerol, dimyristoyl phosphatidic acid, dipalmitoyl phosphatidic, dimyristoyl phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, dimyristoyl phosphatidylserine, dipalmitoyl phosphatidylserine, brain phosphatidylserine, brain sphingomyelin, dipalmitoyl sphingomyelin, and distearoyl sphingomyelin.

The lipid composition of both the initial powdered composition of matter and the resultant liposomes, formed in accordance with the present method, is normally the same. Where the resultant liposomes are intended for <u>in vivo</u> applications (such as drug delivery), then it is normally desirable that the lipid composition have a transition temperature below body temperature. Liposomes composed of phospholipids which have transition temperatures below the characteristic gel-liquid crystalline phase transition temperature of biological membranes, <u>i.e.</u> about 37°C, are considered fluid and those which have transition temperature above 37°C are considered solid. Another consideration in selecting the composition of lipid or lipids for liposome applications

is that alkyl-ether linked lipids (rather than ester linked) are more stable to hydrolysis, and hence alkyl-ether linked lipids for the resultant liposomes may be particularly desirable for therapeutic application.

In addition, other lipid-like substances such as steroids, cholesterol, aliphatic amines or acids such as long chain aliphatic amines or carboxylic acids, long chain sulfates and phosphates, dicetyl phosphate, butylated hydroxytoluene, tocopherol, and isoprenoid compounds may be intermixed with the phospholipid components to confer certain desired and known properties on the initial liposomes and hence the resultant liposomes. Further, synthetic phospholipids containing either altered aliphatic portions, such as hydroxyl groups, branched carbon chains, cycloderivatives, aromatic derivatives, ethers, amides, polyunsaturated derivatives, halogenated derivatives, or altered hydrophillic portions containing carbohydrate, glycol, phosphate, phosphonate, quaternary amine, sulfate, sulfonate, carboxy, amine, sulfhydryl, imidazole groups and combinations of such groups, can be either substituted or intermixed with the phospholipids.

The antifungal polyene macrolides of the present invention include nystatin, amphotericin B, partricin and derivatives thereof such as methyl esters.

These examples are presented to illustrate preferred embodiments and utilities of the present invention and are not meant to limit the present invention unless otherwise stated in the claims appended hereto.

EXAMPLE 1

Preparation and Use of a Pre-Liposomal Nystatin Powder (L-Nys)

A solution of 25 mg nystatin in 25 ml methanol was mixed with a solution of 175 dimyristoylphosphatidylcholine (DMPC) and 75 mg dimyristoylphosphatidylglycerol (DMPG) in 10 ml chloroform. The DMPC:DMPG ration was 7:3 and the nystatin:DMPC+DMPG ration was 1:10. The organic solvents were then evaporated at 40°C under partial vacuum in a rotary evaporator until a dried lipid film was formed. Thirty ml of 2:1 mixture of tertiary butanol and methylene chloride were added to dissolve the dried lipid film. The organic were then evaporated from the solution at 40°C and under partial vacuum to form a lipid residue. The lipid residue was dissolved in tertiary butanol and the solution passed through a 0.2 um filter. The nystatin concentration was measured from an aliquot of the filtrate. The filtrate was frozen by immersion of a container in dry ice-acetone. The frozen material was subjected to overnight lyophilization and a fine pre-liposomal nystatin powder produced.

A 100 mg sample of the fine powder (containing about 10 mg nystatin) was suspended with 10 ml of pyrogen-free saline. When the powder suspension was warmed at 40°C for 2-5 minutes, liposomes were formed therein. As determined by microscopic examination, the suspended materials were 100% liposomes were formed therein. As determined by microscopic examination, the suspended materials were 100% liposomes and no crystals were found. The suspension was centrifuged at 20,000 rpm (40,700 x g) for one hour and the resultant pellet removed and resuspended in saline.

The nystatin remaining in the resuspended pellet was determined to be 70-80 percent of the original amount added, by dissolution in methanol and measurement of optical density at 306 nm. The encapsulation efficiency of the liposomes, as measured after the filtration step, was observed to be > 99%. (No detectable free drug was left after formation of liposomes from the powder). The resuspended pellet was a liposome preparation substantially free of soluble lipids or other materials and was suitable for clinical administration.

EXAMPLE 2

Preparation an Use of a Pre-Liposomal Amphotericin B Powder

Amphotericin B in methanol and phospholipids (DMPC:DMPG, 7:3) in chloroform were mixed together in a ratio of 1:10. The organic solvents were then evaporated at 40°C using a rotary evaporator under vacuum.

Tertiary butanol and methylene chloride in a 1:30-40 ratio were added to solubilize the dried lipid film. The organic solvents were then evaporated.

The residue in the flask was then dissolved in tertiary butanol, warmed to temperatures above 52°C, and filtered through a 0.2 um filter. An aliquot from this filtrate was taken to determine the amphotericin B concentration.

The above mixture was then frozen (using dry ice with acetone) and lyophilized overnight. A fine powder was obtained.

The powder obtained as described above was suspended in pyrogen-free saline. The liposomes did not form until the suspension was warmed in a water bath at about 40°C for about 2-5 minutes. The suspension then formed 100% liposomes (no crystals), as they appeared under a microscope. The suspension was centrifuged at 20,000 rpm for one hour and the pellet removed and resuspended in saline. An aliquot was taken from this final suspension and the amount of amphotericin B incorporated into liposomes quantitated by dissolving in methanol and measuring O.D. at 405 nm. The encapsulation efficiency of drug from the powder to liposomes was 99-100%.

* * * * *

Changes may be made in the elements and methods described herein or in the steps or the sequence of steps of the method described herein without departing from the concept and scope of the invention as defined in the following claims.

WHAT IS CLAIMED IS:

1. A process for producing a powder which forms liposomes comprising an antifungal polyene macrolide upon suspension in an aqueous solution, said process comprising the steps of:

(a) dissolving antifungal polyene macrolide and phospholipids in a quantity of first organic solvent and a quantity of second organic solvent respectively, to form a first solution and a second solution;

(b) mixing the first solution and the second solution to form a mixture;

(c) removing the first organic solvent and the second organic solvent from the mixture to form a residue;

(d) dissolving the residue in a quantity of a third organic solvent to form a third solution;

(e) extracting the third organic solvent from the third solution to leave a remnant;

(f) forming a fourth solution by dissolving the remnant in a solvent consisting essentially of tertiary butanol;

(g) passing the fourth solution through a filter having orifices with diameters of between about 0.1 nm and about 0.5 nm to produce a filtrate; and

(h) lyophlilizing the filtrate to r move the solvent consisting essentially of tertiary butanol.

2. A composition of matter produced essentially by the process of claim 1.

3. A composition of matter produced by a process comprising the steps of:

(a) dissolving antifungal polyene macrolide and phospholipids in a quantity of first organic solvent and a quantity of second organic solvent to form respectively a first solution and a sec nd solution;

(b) mixing the first solution and the second solution to form a first mixture;

(c) removing the first organic solvent and the second organic solvent to form a residue;

(d) dissolving the residue in a quantity of a third organic solvent to form a third solution;

(e) extracting the third organic solvent from the third solution to leave a remnant;

(f) forming a fourth solution by dissolving the remnant in a solvent consisting essentially of tertiary butanol;

(g) passing th fourth solution through a filter having orifices with diameters of between about 0.1 nm and about 0.5 nm to produce a filtrate; and

(h) lyophlilizing the filtrate to remove the solvent consisting essentially of tertiary butanol.

4. The process of claim 1 or composition of matter of claim 3 wherein the antifungal polyene macrolide is nystatin, amphotericin B, partricin or a derivative thereof.

5. The process of claim 1 or composition of matter of claim 3 wherein the antifungal polyene macrolide is nystatin or amphotericin B.

6. The process of claim 1 or composition of matter of claim 3 wherein the antifungal polyene macrolide is amphotericin B.

7. The process of claim 1 or composition of matter of claim 3 wherein the antifungal polyene macrolide is nystatin.

8. The process of claim 1 or the composition of matter of claim 3 wherein the phospholipids are one or more of phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, sphingomyelin and phosphatidic acid.

9. The proc ss of claim 1 or the composition of matter of claim 3 wherein the phospholipids comprise DMPC and DMPG.

10. The process of claim 1 or the composition of matter of claim 3 wherein the phospholipids consist essentially of DMPC and DMPG in 7:3 ratio.

11. The process of claim 1 or the composition of matter of claim 3 wherein the first solvent is methanol.

12. The process of claim 1 or the composition of matter of claim 3 wherein the second solvent is chloroform.

13. The process of claim 1 or the composition of matter of claim 3 wherein step (b) is defined further as:

mixing the first solution and the second solution to form a first mixture having a ratio of anti-fungal polyene macrolide to phospholipid between about 1:5 and about 1:50.

14. The process of claim 1 or the composition of matter of claim 3 wherein step (b) is defined further as:

mixing the first solution and the second solution to form a first mixture having a ratio of anti-fungal polyene macrolide to phospholipid of about 1:10.

15. The process of claim 1 or the composition of matter of claim 3 wherein step (c) is defined further as:

removing the first solvent and the second solvent from the first mixture by subjecting the first mixture to solvent evaporation under reduced pressure and at a temperature between about 35°C and about 45°C.

16. The proces of claim 1 or the composition of matter of claim 3 wherein, prior to the passing step, the fourth solution is clarified by warming to between about 50°C and about 70°C.

17. The process of claim 1 or the composition of matter of claim 3 wherein the third organic solvent comprises tertiary butanol and methylene chloride.

18. The process of claim 1 or the composition of matter of claim 3 wherein the third organic solvent comprises tertiary butanol and methylene chloride in a ratio between about 2:1 and about 1:40.

19. The process of claim 1 or the composition of matter of claim 3 defined further wherein the filter has orifices of about 0.2 nm.

20. The process of claim 1 or the composition of matter of claim 3 wherein the antifungal polyene macrolide and

phospholipids are in a ratio of between about 1:5 and about 1:20.

21. The process of claim 1 or the composition of matter of claim 3 wherein the antifungal polyene macrolide and phospholipids are in a ratio of about 1 to 10.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/03652

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC^4: A 61 K 9/50; A 61 K 31/71

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC^4	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [*]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	US, A, 4663167 (LOPEZ-BERESTEIN et al.) 5 May 1987 see column 5, lines 1-25 --	
A	FR, A, 2390159 (ICI) 8 December 1978 see page 6, example 1; claims --	
A	EP, A, 0087993 (PARFUMS CHRISTIAN DIOR) 7 September 1983 see pages 25,26, example 12; claims --------	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17th February 1989	16 MAR 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 8803652
SA 25325

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 07/03/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4663167	05-05-87	None	
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		DE-A,C 2818655	23-11-78
		JP-A- 53142514	12-12-78
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		CA-A- 1208133	22-07-86

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82